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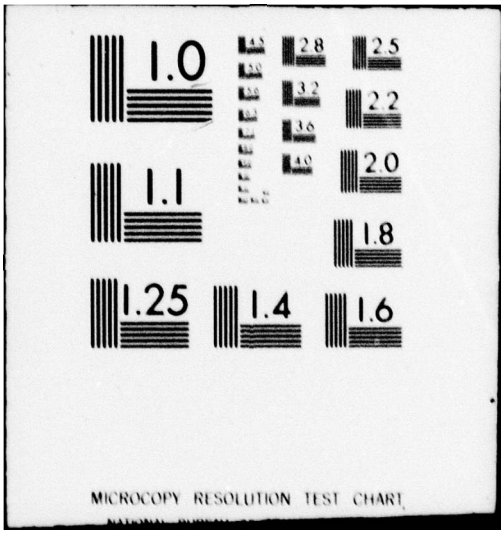
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ABSTRACT

The overall purpose of this project is to understand the means by which phagocytes kill bacteria. The particular goal since the beginning of the report period has been to develop a system to examine the effect of oxygen tension on the respiratory burst of neutrophils.

Previous studies showed that the methods we had used to provide a hypoxic or anoxic environment for neutrophils to work in had led to damage to the cells. During the project period, we were able to devise a technique for incubating neutrophils under hypoxic conditions, and examined the relationship between oxygen tension and O_2^- production by these cells.

SUMMARY

The overall purpose of the project is to understand the means by which phagocytes kill bacteria. The goal set for this project period was to devise a method by which the effect of oxygen tension on neutrophil function could be examined. We were able to accomplish this task through the use of a gassing technique based on submersion of the reaction vessel, and were able to examine phagocytosis and O_2^- production as a function of oxygen tension. The experiments showed that phagocytosis was as efficient under N_2 as under air, a result confirming previous studies. O_2^- production rates by whole neutrophils were constant at oxygen tension levels between room air and 1% O_2, then fell in a linear manner with further declines in oxygen tension.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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BODY OF REPORT

Background

Upon exposure to opsonized bacterial, normal granulocytes undergo changes in their oxidative metabolism (the "respiratory burst") which result in a marked increase in oxygen uptake, a rise in H_2O_2 and superoxdie (O_2^-) production and a tenfold increase in the amount of glucose metabolized via the hexosemonophosphate shunt (1-8). These changes in metabolism are thought to be related to the activation of oxygen-dependent bactericidal mechanisms, since patients whose granulocytes cannot undergo these metabolic alterations (e.g., patients with chronic granulomatous disease or severe glucose-6-phosphate dehydrogenase deficiency) are unusually susceptible to bacterial infections (9,10). Hydrogen peroxide in particular has been implicated in a bactericidal process, since Klebanoff has shown that bacteria are readily killed when incubated with hydrogen peroxide and halide ion in the presence of myeloperoxidase, an enzyme present in large amounts in the primary granules of polymorphonuclear leukocytes (11).

The studies documenting the importance of oxygen to bacterial killing by neutrophils have almost always been conducted in room air. However, infected sites tend to be regions of diminished oxygen tension. While killing in the absence of oxygen can be accomplished by neutrophils (12), the aerobic bactericidal mechanisms are exceptionally powerful--probably more so than the oxygen-independent bactericidal mechanisms --so it is of interest to know the extent to which these mechanisms can operate under conditions of hypoxia.

Results

The following method was used to carry out incubations under defined atmospheres. Siliconized 15 ml Warburg flasks with single sidearms were used as reaction vessels. The cells (2.5×10^7 in 1.5 ml PBS) were placed in the main compartment of the flask, and zymosan (6 mg in 0.5 ml PBS) in the sidearm. The mouth of the flask was stoppered with a syringe port, and the sidearm stoppered with a venting plug cut sufficiently short so the outlet would be under water during the incubation. The venting plug was attached to a source of the desired gas by means of a rubber tube, and the syringe port was pierced by a 20 gauge needle connected to a short length of plastic intravenous tubing. The whole assembly was placed on a low rocking platform designed specifically for this purpose which was placed in the bottom of a Thelco Model 83 water bath and driven by means of an Ames aliquot mixer to which it was connected by a long rod. The water level was such that the needle hub and the venting plug outlet were submerged, and the plastic tubing was arranged so that its remote end was under water as well. The rocking motor was turned on, and the flask was gassed for 7 minutes. Gassing was terminated by first closing the venting plug, then removing the needle from the syringe port; this procedure ensured that the flask would contain the gas at atmospheric pressure. After incubating for 3 more minutes to establish equilibrium between the constituents in the flask and the overlying atmosphere, the contents of the sidearm were tipped into the main compartment, taking care to keep the reaction vessel completely submerged. The flask was placed back on the rocking platform and the incubation continued for the disired length of time. The incubation was terminated by removing the flask from the water bath and immediately placing it in melting ice. The syringe port was then removed and the contents

of the flask, now chilled, were transferred to a homogenizer for further work.

To confirm that the atmospheric composition was maintained in these experiments, measurements were made of O_2^- production by neutrophils incubated under N_2 for 60 minutes. With opsonized zymosan as the respiratory burst stimulus, O_2^- production by whole cells was negligible under nitrogen while values obtained under room air were comparable to those previously reported. These results indicate that this technique is suitable for conducting incubations under defined atmospheres.

Phagocytosis was measured using opsonized zymosan as the test particle. The number of particles associated with the neutrophils (4.8 $\pm$ 0.2 SE particles/cell after 25 minutes of incubation (50 cells were counted to obtain this value)) as measured in air was statistically identical to the value obtained in incubations conducted under N_2. O_2^- production was determined by a previously reported technique, again using opsonized zymosan as the test particle. The rate of O_2^- production under 1% oxygen was 85% that measured in room air. Below 1% oxygen, O_2^- production fell more precipitously, declining linearly with oxygen tension.

Conclusion

These findings indicate that a highly efficient respiratory burst can be mounted by neutrophils at oxygen tensions less than 10% those of arterial blood, and that even under the very hypoxic conditions found at sites of infection, the oxygen-requiring bactericidal mechanisms of the neutrophil are likely to contribute significantly to bacterial killing.

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